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FIELD TRIAL OF ATTENUATED SALMONELLA TYPHI LIVE ORAL  
VACCINE Ty21a IN LIQUID AND ENTERIC-COATED FORMULATIONS  
AND EPIDEMIOLOGICAL SURVEY FOR INCIDENCE OF DIARRHEA  
DUE TO SHIGELLA SPECIES

ANNUAL REPORT

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children, particularly during summer, are due to Shigella and E. coli pathogens. During the summer months in Santa Julia it is likely that Shigella is readily transmitted from child to child by direct contact involving small inocula. Such transmission, which is dependent on personal hygiene practices, is apparently little affected by the widespread availability of potable water in the poblacion. ETEC and EPEC, also commonly associated with diarrhea in summer, are more likely transmitted by contaminated foods. Most households lack refrigerators for food preservation in summer. The high incidence rates of Shigella, ETEC, and EPEC infection make Santa Julia a suitable place for testing the efficacy of vaccines against these agents. (KT)

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## EXECUTIVE SUMMARY

### A FIELD TRIAL COMPARING THE EFFICACY OF TWO DIFFERENT FORMULATIONS OF TY21A LIVE ORAL TYPHOID VACCINE

Inactivated vaccines consisting of killed whole Salmonella typhi organisms inoculated parenterally have been used in military personnel since the Boer War in South Africa. Their routine use in the U.S. Army after the First World War resulted in greatly diminished reported incidences of typhoid fever. Although moderately protective, the killed whole cell vaccines are not satisfactory immunizing agents for use in children in typhoid-endemic areas or among U.S. military personnel because of the frequent and severe adverse reactions that they elicit. A high priority has been given to identifying a well-tolerated yet protective typhoid vaccine.

Attenuated S. typhi strain Ty21a developed by Germanier and Furer is a live oral vaccine candidate. An enteric-coated capsule formulation of Ty21a was evaluated in several large-scale field trials in Santiago, Chile supported by the U.S. Army Medical Research and Development Command and the World Health Organization. Three doses of Ty21a in enteric-coated capsules given within one week provided 69% protection that has endured for at least four years.

In October, 1986, a randomized, controlled field trial was initiated in Area Sur Oriente and Area Norte, Santiago, Chile to compare the relative and absolute efficacy of three doses of Ty21a vaccine given in enteric-coated capsule or liquid formulation. A total of 98,956 pre-randomized schoolchildren, including 63,979 in Area Sur Oriente and 34,977 in Area Norte, received Ty21a vaccine or lactobacilli control preparation in either enteric-coated or liquid formulation. Intensive clinical and bacteriologic surveillance was maintained in the consultorios and hospitals of these areas. Surveillance began in late November, 1986. The code remains unbroken. Preliminary data already show that the liquid formulation is associated with a significantly lower attack rate for typhoid fever than the enteric-coated capsule formulation. Surveillance of this trial must continue to build up sufficient numbers of typhoid cases to be able to have a valid measure of the absolute efficacy conferred by Ty21a in each of these formulations.

### EPIDEMIOLOGIC STUDIES OF DIARRHEA DUE TO SHIGELLA, ESCHERICHIA COLI AND OTHER AGENTS

Diarrheal diseases represent one of the major sources of morbidity for U.S. military personnel deployed in less-developed areas of the world. The lack of adequate sanitation and primitive food and personal hygiene practices in the less-developed world and the lack of immunity of young U.S. adults to the prevalent etiologic (mainly bacterial) agents result in high attack rates of traveler's diarrhea among U.S. military personnel. By studying the epidemiology of diarrheal diseases in young children in less-developed countries, much can be learned that is applicable to

the immunoprophylactic control of diarrheal diseases and dysentery among U.S. soldiers. Furthermore, arguably an accurate measure of the ability of candidate anti-diarrheal (e.g. anti-Shigella) vaccines to protect U.S. military under conditions of natural challenge can be derived by assessing the efficacy of such vaccines in protecting indigenous infants and young children.

Epidemiologic and microbiologic surveillance was initiated in three populations of children in the Santa Julia neighborhood of Area Oriente, Santiago with the broad objective of preparing a field area where the efficacy of vaccines against Shigella, enterotoxigenic E. coli and other diarrheal pathogens can be evaluated. Santa Julia is a densely-populated community of substandard housing. The four arms of the study include:

1) A study of the epidemiology of Shigella and diarrheagenic E. coli in cases and controls in a prospectively followed cohort of 360 children 0-48 months of age whose households are visited twice weekly by public health nurses; 2) A study of the weekly prevalence of Shigella in a subcohort of 120 of the 360 children in the case control study; 3) An exhaustive survey of the etiology of diarrheal disease (including bacterial, viral and protozoal agents) in a cohort of 144 infants followed prospectively from birth for 24 months; and 4) A survey of the frequency of isolation of Shigella and diarrheagenic E. coli from Santa Julia children age 0-48 months who visit the Santa Julia consultorio or who are admitted to the Calvo Mackenna Children's Hospital (that serves Area Oriente) with diarrheal disease.

A summary of the findings of the 4 arms of the epidemiological study is contained in the tables of the annual report. An impressive portion of the episodes of diarrhea in Santa Julia children, particularly during summer, are due to Shigella and E. coli pathogens. During the summer months in Santa Julia it is likely that Shigella is readily transmitted from child to child by direct contact involving small inocula. Such transmission, which is dependent on personal hygiene practices, is apparently little affected by the widespread availability of potable water in the poblacion. The weekly Shigella prevalence study of a subcohort of 120 children of different ages has documented that throughout the year a rather considerable reservoir of Shigella infection exists in the pediatric population of Santa Julia. When warm season arrives, it is from this sizable reservoir that enhanced transmission of Shigella to susceptibles begins.

ETEC and EPEC, also commonly associated with diarrhea in summer, are more likely transmitted by contaminated foods. Most households lack refrigerators for food preservation in summer. The high incidence rates of Shigella, ETEC and EPEC infection make Santa Julia a suitable place for testing the efficacy of vaccines against these agents.

Laboratory studies have only just begun to determine the frequency of isolation of enteroadherent-aggregative E. coli (EA AggEC), a recently-described putative new category of diarrheagenic E. coli. In the short-term these pathogens must be identified by means of the HEP-2 cell assay (carried out in Baltimore). However, two DNA probes have recently been developed at the CVD which appear to be approximately 70% sensitive in identifying EA-AggEC strains from Chile and are highly specific. It is anticipated that when probes of this type are properly standardized they will be transferred to the laboratory in Chile.

## ESTABLISHMENT OF THE FIELD AREA AND THE SUPPORTING LABORATORY

### Selection of the Field Area

Santa Julia is a neighborhood of low socioeconomic level and mostly ramshackle housing located in the Eastern administrative area of Santiago (Area Oriente). Such an area is referred to as a "poblacion". The total population of poblacion Santa Julia is 133,909 inhabitants contained within a surface area of 12.3 km<sup>2</sup>; the population density is 3.27 persons/km<sup>2</sup>. Approximately 31% of the population is below 15 years of age. The cohort of 360 children < 5 years of age followed prospectively to study the epidemiology of diarrheal disease due to Shigella and E. coli was recruited in September, 1986 and surveillance began in earnest in October. A young pediatrician was hired to provide primary pediatric care to children in the two cohorts under prospective surveillance. The Director of the Consultorio Santa Julia was very cooperative with his limited resources. Although space within the consultorio was at a premium, the Director identified an area where we were permitted to construct a new room specifically to house our field staff and activities. In addition, he temporarily provided us part-time use of one of the treatment rooms where children in the cohorts could be examined and cared for when they required medical attention or well baby care. In this way, the mothers and children participating in the study do not have to wait long to receive care but are seen almost immediately by the project physician. (At some point in the future it will be necessary to prepare a separate examination room specifically to house the project's clinical care activities).

### Acquisition of Laboratory Space

In order to perform the extensive laboratory studies that support the field studies, a collaboration was undertaken with Dr. Valeria Prado, Head of the Microbiology Unit of the University of Chile Faculty of Medicine, Oriente. In anticipation of this collaboration, Dr. Prado acquired significant new laboratory space in another building. Following renovation of this space, the Microbiology Unit moved to the new area in September, 1986.

Equipment for the laboratory was purchased in the U.S.A. and shipped to Santiago. The arrival of the equipment was followed by a series of consultants from the Center for Vaccine Development of the University of Maryland who traveled to Santiago to instruct the Chilean collaborators and technicians in the use of the equipment and to establish the various immunoassays and DNA probe techniques.

The identification of the various categories of diarrheagenic E. coli represents an exceedingly ambitious aspect of the project. In theory, the DNA probes provide a single technique that can be applied to identify all but one (enteroadherent-aggregative E. coli) of the recognized categories of diarrheagenic E. coli. However, to transfer this technology to Santiago meant overcoming two major obstacles: the first was to modify the signal identifying a positive test from a radioisotopic to a colorimetric signal; the second was to train a Chilean team of excellent microbiologists who, however, had no prior experience in molecular biology. This was accomplished by sending to Santiago a CVD molecular biologist who is fluent in Spanish and has considerable experience in the less-developed world.

FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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1. Weekly prevalence of Shigella carriage in 120 children 0 - 48 months of age . . . . .

I. A FIELD TRIAL COMPARING THE EFFICACY OF TWO DIFFERENT  
FORMULATIONS OF TY21A LIVE ORAL TYPHOID VACCINE

The transmission of typhoid fever is favored by circumstances where food and water vehicles can readily become contaminated. Historically, typhoid fever was a major problem of military personnel in the 19th century, and was rampant, for example, among U.S. troops in Cuba during the Spanish-American War (1). Inactivated vaccines consisting of killed whole Salmonella typhi organisms inoculated parenterally have been used in military personnel since the Boer War in South Africa. Their routine use in the U.S. Army after the First World War resulted in greatly diminished reported incidences of typhoid fever (2). A series of randomized, placebo-controlled field trials carried out in the 1950s and 1960s under the auspices of the World Health Organization established unequivocally that the acetone-inactivated and heat-phenol-inactivated whole cell typhoid vaccines provide significant protection for at least three years (up to seven years in one trial in Guyana) (3-6). Several of the aforementioned vaccines used in these controlled field trials were prepared at the Walter Reed Army Institute of Research (7). Although moderately protective, the killed whole cell vaccines are not satisfactory immunizing agents for use in children in typhoid-endemic areas or among U.S. military personnel because of the frequent and severe adverse reactions that they elicit. In controlled double-blind studies, the killed whole cell parenteral vaccines cause fever in approximately 25% of recipients, incapacitating malaise leading to

absenteeism in 15-20% and notable local adverse reactions in about 50% (5,8,9). Consequently, a high priority has been given to identifying a well-tolerated yet protective typhoid vaccine.

Attenuated S. typhi strain Ty21a developed by Germanier and Furer (10) was proposed by those investigators as a possible live oral vaccine candidate. In preliminary studies in North American volunteers, Ty21a, even in doses as high as  $10^{11}$  viable organisms, was well-tolerated (11). Furthermore, in experimental challenge studies in volunteers Ty21a was highly protective (11).

Based on the encouraging results in North American volunteers, the first field trial of efficacy of Ty21a was carried out in 6-7 year old schoolchildren in Alexandria, Egypt where approximately 16,000 children received three doses of vaccine (on Monday, Wednesday and Friday of one week) and 16,000 received placebo (12). Lyophilized vaccine was reconstituted in the field and children ingested the liquid suspension several minutes after chewing a 1.0 gm  $\text{NaHCO}_3$  tablet to neutralize gastric acid. After three years of surveillance 22 culture-confirmed cases were recorded in the placebo group, while only one case occurred in the vaccine group (96% vaccine efficacy) (12).

Subsequently a more practical formulation of Ty21a was developed consisting of lyophilized vaccine in enteric-coated capsules. This practical formulation was evaluated in several large-scale field trials in Santiago, Chile supported by the U.S. Army Medical Research and Development Command and the World Health Organization (3,13,14). In the second trial, which was initiated in Area Occidente in August, 1983, the enteric-coated capsule

formulation was compared with a commercial formulation consisting of lyophilized vaccine and  $\text{NaHCO}_3$  in gelatin capsules (14). The enteric-coated capsule formulation was significantly more protective than the gelatin capsule/ $\text{NaHCO}_3$  formulation. Three doses of Ty21a in enteric-coated capsules given within one week provided 69% protection that has endured for at least four years (Table 1) (14).

The 69% vaccine efficacy for at least four years conferred by three doses of Ty21a in enteric-coated capsules given within one week is quite a credible performance by a vaccine. Nevertheless, it is notably less than the 96% protection conferred by Ty21a in Alexandria, Egypt where a liquid formulation of vaccine was used. There are many other possible explanations to account for the differences in efficacy between the Alexandria and Area Occidente trials, including human genetics affecting immune response to vaccine, antigenic differences between prevalent S. typhi strains, possible distinct modes of transmission that might affect inoculum size and thereby vaccine efficacy, and differences in surveillance methods (active in Alexandria, passive in Occidente). Nonetheless, the difference in formulation of vaccine between the two trials is the one variable that could be directly tested. Accordingly, in October, 1986, a randomized, controlled field trial was initiated in Area Sur Oriente and Area Norte, Santiago, Chile to compare the relative and absolute efficacy of three doses of Ty21a vaccine given in enteric-coated capsule or liquid formulation.

#### Trial Design

In October and November, 1986, a total of 98,956 pre-randomized

schoolchildren, including 63,979 in Area Sur Oriente and 34,977 in Area Norte, received Ty21a vaccine or placebo in either enteric-coated or liquid formulation. A total of 84,836 children received all three scheduled doses of vaccine or placebo within the eight day immunization period. The Area Norte participants represent children who entered school subsequent to the initiation of the 1982 field trial in Area Norte.

The "liquid" formulation consisted of two sachets, one containing a single dose of  $1-3 \times 10^9$  lyophilized vaccine organisms (or lyophilized lactobacilli serving as the control preparation) and the other containing a buffer. One sachet of buffer and one of vaccine (or lactobacilli control preparation) was emptied into a disposable cup, 100 ml of water was added, the contents were stirred and the suspension was ingested by the schoolchild.

Because of ethical considerations, the size of the control group was kept relatively small; only one-eighth of the participating children received the control preparation. In order to maintain blindness, there were eight separate coded liquid preparations, of which one was lactobacilli. Similarly, among the eight coded enteric-coated capsule preparations, one contained lactobacilli.

#### Results of Surveillance

In Area Sur Oriente approximately 90% of all health care visits take place in health centers (consultorios) of the National Health Service, the corresponding percent for Area Norte is 85%. Accordingly, intensive clinical and bacteriologic surveillance was

maintained in the consultorios and hospitals of these areas. Two 6 ml blood cultures 30 minutes apart were obtained from all children presenting as outpatients to consultorios or hospitals with a clinical syndrome suspect of being typhoid fever. From hospitalized pediatric inpatients with presumed typhoid fever, three blood cultures were obtained. Only culture-confirmed cases were utilized in computation of incidence rates, data analysis and statistical comparisons.

Surveillance began in late November, 1986. Cumulative results of surveillance which include the period of observation from 11/1/86 to 10/31/87 are summarized in Table 2. The code remains unbroken. Thus it is not possible to calculate absolute vaccine efficacy. Nevertheless, although the recipients of placebo cannot be identified, it is known who received the liquid and who received the enteric-coated capsule formulations of Ty21a. Consequently, it is possible even at this time to compare the relative efficacy of the two different formulations, recognizing that approximately one-eighth of the recipients of each of these two formulations received lactobacilli control preparation, rather than vaccine. As shown in Table 2, the preliminary data already show that the liquid formulation is associated with a significantly lower attack rate for typhoid fever than the enteric-coated capsule formulation. Surveillance of this trial must continue to build up sufficient numbers of typhoid cases to be able to have a valid measure of the absolute efficacy conferred by Ty21a in each of these formulations.

## II. EPIDEMIOLOGICAL STUDIES OF DIARRHEA DUE TO SHIGELLA, ESCHERICHIA COLI AND OTHER AGENTS

Diarrheal diseases represent one of the major sources of morbidity for U.S. military personnel deployed in less-developed areas of the world (15,16). The lack of adequate sanitation and primitive food and personal hygiene practices in the less-developed world and the lack of immunity of young U.S. adults to the prevalent etiologic (mainly bacterial) agents result in high attack rates of traveler's diarrhea among U.S. military personnel. In less-developed areas, incidence rates of diarrheal disease are high among children in the first three years of life (16-19). One consequence of these repeated infections in early childhood is the acquisition of immunity that results in a low incidence of diarrheal disease in indigenous adults and older children (16,20,21). However, when U.S. adults travel to less-developed areas they immunologically resemble infants and young children, not indigenous adults. Consequently, by studying the epidemiology of diarrheal diseases in young children in less-developed countries, much can be learned that is applicable to the immunoprophylactic control of diarrheal diseases and dysentery among U.S. soldiers. Furthermore, arguably an accurate measure of the ability of candidate anti-diarrheal (e.g. anti-Shigella) vaccines to protect U.S. military under conditions of natural challenge can be derived by assessing the efficacy of such vaccines in protecting indigenous infants and young children.

### The Four Arms of the Epidemiological Studies in Santa Julia

Epidemiologic and microbiologic surveillance was initiated in

three populations of children in the Santa Julia neighborhood of Area Oriente, Santiago with the broad objective of preparing a field area where the efficacy of vaccines against Shigella, enterotoxigenic E. coli and other diarrheal pathogens can be evaluated. Santa Julia is a densely-populated community of substandard housing. The four arms of the study include:

1. A study of the epidemiology of Shigella and diarrheagenic E. coli in cases and controls in a prospectively followed cohort of 360 children 0-48 months of age whose households are visited twice weekly by public health nurses.
2. A study of the weekly prevalence of Shigella in a subcohort of 120 of the 360 children in the case control study.
3. An exhaustive survey of the etiology of diarrheal disease (including bacterial, viral and protozoal agents) in a cohort of 144 infants followed prospectively from birth for 24 months.
4. A survey of the frequency of isolation of Shigella and diarrheagenic E. coli from Santa Julia children age 0-48 months who visit the Santa Julia consultorio or who are admitted to the Calvo Mackenna Children's Hospital (that serves Area Oriente) with diarrheal disease.

Active Surveillance of a Cohort for Shigella and E. coli Diarrhea

A total of 360 children were enrolled into this arm of the study, some of whom have been lost to follow-up by out-migration from Santa Julia. Public health field nurses working on the project visit the houses of participating children twice weekly to detect cases of diarrheal disease. Diarrhea is defined as an overt change in the normal stool pattern of the child characterized by an

increase in the frequency and decrease in the consistency of stools to an unformed state noted by the child's caretaker; this must comprise passage of at least three loose stools in a 24 hour period. If a child has diarrhea, a pre-determined, matched control child in the cohort is visited to ascertain that the control is without diarrhea. A stool specimen or rectal swab is obtained on two consecutive days from the child with diarrhea and from his/her age-matched control and examined for Shigella and the different categories of diarrheagenic E. coli.

Stool specimens are transported to the laboratory in tubes containing buffered glycerol saline transport medium (the preferred transport for Shigella) (22) and are immediately plated onto MacConkey's, Salmonella-Shigella, and xylose-lysine-desoxycholate agar. Lactose-negative colonies are picked to identify Shigella and enteroinvasive Escherichia coli, the latter by means of a DNA probe(23). Lactose-positive colonies are examined to detect enterotoxigenic E. coli(24), enteroinvasive E. coli(23), enteropathogenic E. coli(25), and enterohemorrhagic E. coli(26). E. coli strains are also examined to record their pattern of adherence to HEp-2 cells (24).

Table 3 summarizes by month the number of children under surveillance, the episodes of diarrhea that occurred, the number of matched healthy controls obtained, the total number of coprocultures obtained from each group, and the isolation of Shigella from cases and controls. It is obvious that in this first year of surveillance diarrhea in young children showed a prominent seasonality with approximately twice as many episodes occurring in

the warm season from December, 1986 through March, 1987.

Similarly, isolation of Shigella was maximal during the warm months and was found significantly more often in cases than during the winter months (Table 3). The difference in isolation rate of Shigella from cases (12.9%) versus controls (28%) was highly significant ( $p < 0.0001$ ).

Evidence of the diligence and tenacity of the study nurses working in the field is demonstrated by the fact that a matched control child was successfully cultured for 467 of the 487 episodes of diarrhea (96%) that occurred during this year of surveillance. Similarly, of the total 1908 coprocultures that should have been obtained from these children (two per child on consecutive days), including 974 from cases and 934 from controls, 1866 (98%) were in fact successfully obtained.

Table 4 analyzes the occurrence of Shigella infections by age group. Displayed are the child months of observation by age group, the number of episodes of diarrhea, the mean episodes per 12 child months of observation, and the occurrence of all Shigella for the period from November 1, 1986 until October 31, 1987. The rate of diarrheal illness, expressed as the incidence per 12 child months of observation, is highest in the first year of life and steadily decreases thereafter. The incidence of Shigella infection is similar for all the age groups except the 36-47 month age group where the incidence, 0.29 cases per 12 child months of observation, is approximately twice as high as in the other groups. The percent of diarrheal episodes due to Shigella is lowest in the 0-11 month age group (5 of 67, 7.5%) and highest in the 36-47 month olds where

25% of all episodes (23 of 92) were due to Shigella.

This prospective cohort study is also meant to explore Santa Julia as a possible site to evaluate candidate vaccines against enterotoxigenic E. coli and other diarrheagenic E. coli in controlled field trials. During the first year of operation of this research contract, a considerable effort was expended to introduce DNA probe technology that employs a colorimetric (biotin-labelled) rather than a radioisotopic signal, into the Microbiology Unit of the University of Chile, Sede Oriente. DNA probes were considered the method of choice to identify the different categories of diarrheagenic E. coli. The progress made in transfer of this technology, a description of the hurdles that had to be overcome and the solutions devised are contained in another section of this report. Biotinylated probes to identify enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), enterohemorrhagic E. coli (EHEC), and enterotoxigenic E. coli (ETEC) that elaborate heat-labile enterotoxin (LT) have been successfully introduced and have been utilized to test E. coli strains isolated from cases and controls in this cohort in the period 11/1/86 to 10/31/87. However, biotinylated, or comparable non-radioactive probe methods to detect E. coli strains that possess genes to elaborate human or porcine heat-stable enterotoxins, have not yet been successfully introduced into the laboratory in Chile. Nevertheless, approximately 90% of the E. coli strains from the period of 12/1/86 to 8/31/87 have been tested at the Center for Vaccine Development in Baltimore using the radioisotope

-(<sup>32</sup>P)-labelled STh probe.

Table 5 shows the identification of diarrheagenic E. coli by probe technique from cases of diarrhea and controls tested during the period from November 1, 1986 to October 31, 1987. ETEC with the capacity to elaborate LT was the most common diarrheagenic E. coli identified by DNA probe. Although E. coli strains from only nine months of surveillance have been tested so far with a <sup>32</sup>P-labelled probe for STh, strains having genes coding for this toxin were commonly found in samples from children with diarrhea. EPEC were the next most common category of diarrheagenic E. coli. EIEC and EHEC were less common. For none of these pathogens was the isolation rate significantly greater among cases than among controls with the data available from only one year of surveillance (only 10 months for STh). Other studies of diarrheal disease in Latin America have also shown similar rates of isolation of ETEC from cases as compared with controls (28,29).

Also shown in Table 5 is the rate of isolation of E. coli strains that hybridize with a DNA probe that detects genes that encode the property of diffuse adherence to HEp-2 cells (27). Such strains, which were isolated somewhat more often in the warm months, were identified at a similar rate among cases (24.5%) and controls (16.8%).

The E. coli pathogens detected by DNA probes are analyzed by age group in Table 6. In this more appropriate analysis of EPEC, the data suggest that in the first 12 months of life EPEC may indeed an important cause of infant diarrhea associated more frequently with cases (8 of 55, 14.5%) than controls (3 of 53,

5.7%) ( $p = 0.058$ , Fisher's test, 1 tail), as has been previously found in health center based studies in Santiago (27).

#### Weekly Prevalence of Shigella Carriage in A Subcohort

In order to quantitate the magnitude of the reservoir of Shigella, in particular to determine the prevalence of subclinical infections, a subcohort of 120 children of all ages were randomly selected from among the cohort of 360 prospectively followed children. These 120 children had weekly stool cultures collected to detect Shigella. Results of these weekly prevalence cultures are shown in Figure 1. A total of 52 of the 120 children had positive cultures for Shigella, 38 of which (73%) were subclinical infections, demonstrating that the reservoir of Shigella is far greater than estimated on the basis of measurement of incidence of clinical illness alone. While most infections were documented during the warm months of the year (December through March), subclinical infections were also recorded in the cool months, suggesting that in this way Shigella may carry over to the next warm season when conditions are again favorable for enhanced transmission. As seen in Figure 1, many asymptotically-infected children shed Shigella for several weeks consecutively.

#### Newborn Prospective Cohort Study of the Etiology of Diarrhea

Commencing in March, 1987, 12 newborn infants each month were entered into the infant cohort study. The enrollment of infants into this study for the period up to October 31, 1987 is shown in Table 7; 52% are male and 44% are first-borns. Table 7 also shows the occurrence of diarrhea in this cohort by calendar month and in relation to the age of the children at the time of onset of

diarrhea.- Also shown are the number of days of diarrheal disease morbidity in the cohort, the mean duration of diarrhea and the percentage of cases in which the duration of diarrhea exceeded 14 days. For each infant with diarrhea, an age-matched healthy control infant is selected to be cultured as well. The stool cultures from this arm of the study have not yet been tested for diarrheagenic E. coli. Nevertheless, the specimens have been submitted to routine coproculture, culture for Campylobacter and for detection of rotavirus by ELISA (30). These results for the infants with diarrhea, by age group, are shown in Table 8.

#### Hospital and Health Center-Based Surveillance

In addition to active prospective surveillance of a cohort of 360 children from 0-48 months of age involving twice weekly household visits to study the epidemiology of Shigella and diarrheagenic E. coli infection, passive surveillance is being maintained at the Consultorio Santa Julia and at the Calvo MacKenna Children's Hospital (which serves Area Oriente). At the consultorio this involves obtaining stool cultures from all children with diarrhea less than 48 months of age who present to the consultorio to detect Shigella and diarrheagenic E. coli.

At the hospital, the records of children < 5 years of age admitted with acute diarrheal disease are reviewed daily (Monday to Friday) to identify children from Santa Julia. These children are cultured to detect Shigella and diarrheagenic E. coli. Both in the hospital and at the consultorio any children from the cohort of 360 are identified to record those episodes of diarrhea that were sufficiently severe to require the parent to take the child to a

health care facility or to require hospitalization.

The isolation of Shigella by month from children with diarrhea seen at the consultorio and in the hospital is summarized in Table 9. Virtually an identical pattern of seasonality of isolation of Shigella is seen as was found in the active surveillance cohort study. The relative frequency of occurrence of Shigella by species is shown in Table 10, comparing the active surveillance cohort, health center and hospital isolations. Irrespective of the sampling site, the relative proportion of isolations of Shigella by serogroup were virtually identical, 53-59% were S. sonnei and 42-47% were S. flexneri, the small remainder were S. boydii.

It has been a common supposition among epidemiologists that Shigella are isolated from a somewhat greater proportion of children with diarrhea seen in hospitals and health centers than with diarrhea detected by active household surveillance because the first two represent a more severe spectrum of clinical illness and Shigella is more prone to cause diarrheal illness of greater severity. Heretofore it was not readily possible to critically test this hypothesis from other published studies undertaken elsewhere. Constraints of earlier studies include: 1) similar populations in the same geographic area were not tested; 2) different bacteriologic techniques were utilized in hospital versus field surveillance; 3) the populations were not of similar age structure for comparison. In the Santa Julia study we were able to overcome these limitations. In Table 11, the relative frequency of isolation of Shigella from coprocultures of children with diarrhea, by age group, is compared for children from the active surveillance

- cohort, from the consultorio and from the hospital. Indeed, some significant differences in rate of isolation were found. For example, among children in the first year of life with diarrheal illness, Shigella was significantly more often isolated from children of that age admitted to hospital with diarrheal disease than among those with diarrhea detected by household visits or at the consultorio. Similarly, among toddlers (i.e 12-23 month olds) admitted to hospital with diarrheal disease, Shigella was more often isolated (4 of 14, 28.6%) than it was among toddlers with diarrhea detected by household surveillance (11 of 157, 7.0%) ( $p = 0.023$ , Fisher's exact test, 2 tails).

In Table 12 the isolation of Shigella is analyzed in relation to the clinical form of diarrheal disease. In the presence of overt dysentery, characterized by the presence of blood and mucous in diarrheal stools, Shigella was isolated significantly more often than from children who had simple diarrhea without blood and mucous.

The isolation of the different categories of diarrheagenic E. coli as detected by DNA probes from cultures of children with diarrhea seen at the consultorio or the hospital is shown in Table 13.

#### Comment

The mean number of episodes of diarrhea per child per year in Santa Julia is notably less than has been recorded for rural village children in Bangladesh prospectively for diarrheal disease (17) or for young children in rural Northeastern Brazil (the least-developed part of that country) (18). Nevertheless, an

-- impressive portion of the episodes of diarrhea in Santa Julia children, particularly during summer, are due to Shigella and E. coli pathogens. These data corroborate the seroepidemiologic studies of Levine et al (21) reported several years ago which showed that among Santiago children 3-5 years of age, the prevalence and mean titer of LT antitoxin (a measure of past infection with LT-producing ETEC) was as high as that found in Bangladeshi children of the same age. During the summer months in Santa Julia it is likely that Shigella is readily transmitted from child to child by direct contact involving small inocula. Such transmission, which is dependent on personal hygiene practices, is apparently little affected by the widespread availability of potable water in the poblacion. The weekly Shigella prevalence study of a subcohort of 120 children of different ages has documented that throughout the year a rather considerable reservoir of Shigella infection exists in the pediatric population of Santa Julia. When warm season arrives, it is from this sizable reservoir that enhanced transmission of Shigella to susceptibles begins.

ETEC and EPEC, also commonly associated with diarrhea in summer, are more likely transmitted by contaminated foods. Most households lack refrigerators for food preservation in summer. The high incidence rates of Shigella, ETEC and EPEC infection make Santa Julia a suitable place for testing the efficacy of vaccines against these agents.

Laboratory studies have only just begun to determine the frequency of isolation of enteroadherent-aggregative E. coli (EA\_AggEC), a recently-described putative new category of diarrheagenic

E. coli (27,31,32). In the short-term these pathogens must be identified by means of the HEP-2 cell assay (carried out in Baltimore). However, two DNA probes have recently been developed at the CVD which appear to be approximately 70% sensitive in identifying EA-AggEC strains from Chile and are highly specific. It is anticipated that when probes of this type are properly standardized they will be transferred to the laboratory in Chile.

### III. ESTABLISHMENT OF THE FIELD AREA AND THE SUPPORTING LABORATORY

#### Selection of the Field Area

Santa Julia is a neighborhood of low socioeconomic level and mostly ramshackle housing located in the Eastern administrative area of Santiago (Area Oriente). Such an area is referred to as a "poblacion". The total population of poblacion Santa Julia is 133,909 inhabitants contained within a surface area of 12.3 km<sup>2</sup>; the population density is 3.27 persons/km<sup>2</sup>. Approximately 31% of the population is below 15 years of age. The birth rate in Santa Julia is approximately 19.8. Almost the entire population of Santa Julia has access to bacteriologically-monitored water, either from a tap within the household or from one located within a few yards of the dwelling.

Approximately 96% of the population of Santa Julia has its health care provided by the National Health Service, mainly through a health center, Consultorio Santa Julia, located in the poblacion. During August and September, 1986, mothers of children less than five years of age living in Santa Julia were recruited to have their children enter one of three cohorts to participate in prospective studies of diarrheal disease. In August, 1986, an

experienced public health nurse, Ms. Marisol Cayazzo was hired to supervise a team of other nurses and auxiliaries working in the field. In the course of these two months, Dr. Ferreccio and Ms Cayazzo trained the new team in epidemiologic field methods of prospective household surveillance, in proper procedures for collecting stool specimens, and in home management of diarrheal disease.

The cohort of 360 children < 5 years of age followed prospectively to study the epidemiology of diarrheal disease due to Shigella and E. coli was recruited in September, 1986 and surveillance began in earnest in October. A young pediatrician was hired to provide primary pediatric care to children in the two cohorts under prospective surveillance. The Director of the Consultorio Santa Julia was very cooperative with his limited resources. Although space within the consultorio was at a premium, the Director identified an area where we were permitted to construct a new room specifically to house our field staff and activities. In addition, he temporarily provided us part-time use of one of the treatment rooms where children in the cohorts could be examined and cared for when they required medical attention or well baby care. In this way, the mothers and children participating in the study do not have to wait long to receive care but are seen almost immediately by the project physician. (At some point in the future it will be necessary to prepare a separate examination room specifically to house the project's clinical care activities).

#### Acquisition of Laboratory Space

In order to perform the extensive laboratory studies that

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support the field studies, a collaboration was undertaken with Dr. Valeria Prado, Head of the Microbiology Unit of the University of Chile Faculty of Medicine, Oriente. In anticipation of this collaboration, Dr. Prado acquired significant new laboratory space in another building. Following renovation of this space, the Microbiology Unit moved to the new area in September, 1986.

Equipment for the laboratory was purchased in the U.S.A. and shipped to Santiago. The arrival of the equipment was followed by a series of consultants from the Center for Vaccine Development of the University of Maryland who traveled to Santiago to instruct the Chilean collaborators and technicians in the use of the equipment and to establish the various immunoassays and DNA probe techniques. Mr. Ben Tall and Ms. Linda LaPierre of the Enteric Microbiology Section of the Center for Vaccine Development (CVD) set up the work flow patterns for the high volume bacteriology studies, organized large-scale media production, established quality control procedures and taught the system of specimen cataloging and storage. Dr. Genevieve Losonsky and Mr. Charles Young of the Applied Immunology Section of the CVD instructed the Chilean laboratory team in the performance of immunoassays to detect rotavirus and enteric adenovirus and in the operation of the ELISA washer and reader.

Dr. Steven Wasserman, who heads the Biostatistics and Data Processing Unit within the CVD (and who is fluent in Spanish), designed two critical data bases. One data base contains all relevant demographic information about the children, notation of their episodes of diarrhea, the clinical features of the illness,

of learning any totally new assay, as well as those involved in transferring a highly specialized technology. Dr. James Kaper provided instruction in the theoretical basis of the assays during his stay, while Kevin Maher over the next four weeks taught the actual techniques. The basic procedures were well-grasped by the personnel involved, Dr. Leo Maggi and T.M. Juan Martinez. However, difficulties inherent in the protocol surfaced shortly thereafter. The original protocol was developed and written by someone with extensive experience in the use of DNA probes; consequently, many simple details were taken for granted. Another problem stemming from the lack of experience was that procedures that were unsuccessful were repeated without modification; nor were steps initiated to determine the reason for the suboptimal results. These problems were remedied by sending to Santiago a CVD molecular biologist, Dr. Mary Baldini, who is fluent in Spanish and has considerable experience in the less-developed world. She undertook a series of protocols to diagnose the source of the suboptimal results and was able in fairly short time to rectify the situation. Blots done previous to her arrival were often difficult to read and complicated by high background; in contrast, the blots done using her revised protocols were clean and readable.

In addition to solving the immediate problems of the DNA probe technique, Dr. Baldini and her Chilean collaborators instituted research protocols to further simplify and notably economize the DNA probe assays as performed in the Microbiology Unit in Santiago. Heretofore one major drawback to using biotinylated probes has always been the expense of the proteinase K used in

preparing the filters (although this is relatively a less significant problem in Chile where  $^{32}\text{P}$  is extremely expensive). The protocol of Haas and Fleming (34) uses 30 ml of 200 ug/ml proteinase K per filter (\$3.55/filter); Sethabutr et al (35) do not specify a volume but use a concentration of 1 mg/ml (at least \$1/filter); the original Santiago protocol used 8 ml of 1 mg/ml proteinase K per filter (\$4.00/filter). The first modification to the protocol designed by Dr. Baldini involved notably diminishing the concentration of proteinase K used such that the cost was reduced to only \$0.25 per filter. In addition, it was found that a much lower concentration of probe DNA could be used and that the hybridization solution could be stored at ambient temperatures up to 35°C or -20°C and re-used until the probe DNA was used up. As a consequence of Dr. Baldini's 12 month stay in Santiago devoting full time to the project, training the Chilean microbiologists and serving as an on-site "trouble-shooter", the DNA probe technology is functioning smoothly and several additional DNA probes will soon be introduced into the Microbiology Unit.

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**Table 1.** Duration of the efficacy conferred by three doses of the enteric-coated capsule formulation of Ty21a live oral vaccine given within one week in Area Occidente, Santiago, Chile

	<u>Vaccine*</u> <u>(22,170)</u>	<u>Placebo*</u> <u>(21,906)</u>
<b>Year 1</b> <b>(9/83-8/84)</b>		
Cases	7	24
Incidence/10 <sup>5</sup>	31.6	109.6
Efficacy	71.2	-
<b>Year 2</b> <b>(9/84-8/85)</b>		
Cases	8	20
Incidence/10 <sup>5</sup>	36.1	91.3
Efficacy	60.5	-
<b>Year 3</b> <b>(9/85-8/86)</b>		
Cases	8	24
Incidence/10 <sup>5</sup>	36.1	109.6
Efficacy	67.1	-
<b>Year 4</b> <b>(9/86-8/87)</b>		
Cases	4	18
Incidence/10 <sup>5</sup>	18.0	82.2
Efficacy	78.1	-
<b>Total, Years 1-4</b> <b>9/83-8/87</b>		
Cases	27	86
Incidence	121.7	392.6
Efficacy	69.0	-

\* 3 doses, 1-2 days between doses

a vs b,  $p < 0.00001$ , Chi square

Table 2. Preliminary results for the period of November, 1986 to October, 1987 of a field trial in Area Sur Oriente and Area Norte assessing the efficacy of Ty21a vaccine in liquid or enteric-coated capsule formulations, in comparison with placebo.

Letter codes of			
Liquid	No.	No.	Incidence
<u>Preparations</u>	<u>Children</u>	<u>Cases</u>	<u>per 105</u>

B,H,I,J,L,O,P & Q	43,712	10	22.9a
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Letter codes of			
Enteric-coated capsule			
<u>Preparations</u>			

F,G,K,M,N,R,S & T	41,124	29	70.5b
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a vs b,  $p < 0.002$

Note — one letter code of liquid and one of enteric-coated capsule formulation, i.e. one-eighth of each group, represents placebo recipients.

**Table 3.** Isolation of Shigella from cases of diarrheal disease and from age-matched controls. Cohort study of 360 children < 5 years of age, Santa Julia, Santiago, November 1, 1986 to October 31, 1987

<u>Month</u>	<u>No. of Children in the Cohort</u>	<u>Cases of Diarrhea</u>	<u>Diarrhea Cases due to Shigella</u>	<u>Matched Healthy Controls</u>	<u>Shigella in Healthy Controls</u>
1986					
November	359	56	10 (17.8%)	56	0 (0%)
December	356	53	12 (22.6%) <sup>a</sup>	53	3 (5.7%)
1987					
January	353	53	15 (28.3%) <sup>b</sup>	53	2 (3.8%)
February	347	40	6 (15.0%) <sup>c</sup>	40	3 (7.5%)
March	342	60	7 (11.7%)	56	2 (3.0%)
April	339	56	3 (5.4%)	54	0 (0%)
May	337	37	2 (5.4%)	34	0 (0%)
June	337	22	0 (0%) <sup>d</sup>	21	2 (9.5%)
July	335	29	2 (6.9%) <sup>e</sup>	24	1 (4.2%)
August	335	28	2 (7.1%) <sup>f</sup>	27	1 (3.7%)
September	335	27	1 (3.7%)	23	0 (0%)
October	335	26	3 (11.5%)	26	0 (0%)
Total		487	63 (12.9%) <sup>g</sup>	467	14 (3.0%) <sup>h</sup>

Rate of isolation of Shigella from patients in summer months (a + b + c) versus winter months (d + e + f),  $p = 0.0001$

Isolation of Shigella among cases versus controls (g vs. h),  $p < 0.00001$

Table 4. Isolation of Shigella species from diarrhea cases and controls by age. Cohort of 360 children under prospective surveillance, November 1, 1986 to October 31, 1987.

Age Group (months)	Clinical Status	Diarrheal Episodes	Onset months of		S. sonnei	S. flexneri	S. boydii	S. dysenteriae	Diarrhea/ 12 Child Months of Observation		Shigella/ 12 Child Months of Observation	
			Observation	Observation								
0-11	Diarrhea	67	386	4	0	0	1	0	2.08	0.15	-	-
	Controls	-	-	1	0	0	0	0	-	-	-	-
12-23	Diarrhea	157	1065	10	2	0	0	0	1.77	0.13	-	-
	Controls	-	-	1	0	0	0	0	-	-	-	-
24-35	Diarrhea	134	1155	9	6	2	0	0	1.39	0.18	-	-
	Controls	-	-	3	0	1	0	0	-	-	-	-
36-47	Diarrhea	92	875	10	13	0	0	0	1.26	0.32	-	-
	Controls	-	-	1	4	1	0	0	-	-	-	-
≥ 48	Diarrhea	37	629	1	5	0	0	0	0.70	0.11	-	-
	Controls	-	-	0	2	0	0	0	-	-	-	-
Totals	Diarrhea	487	4110	34	26	3	0	0	1.42	0.18	-	-
	Controls	-	-	6	6	2	0	0	-	-	-	-

Table 5. Isolation of diarrheagenic *E. coli* by DNA probe methodology in cases of diarrhea and controls in a cohort of 360 children 0 - 48 months of age followed prospectively by active surveillance involving twice weekly household visits. November 1, 1986 to October 31, 1987.

Month	Controls		Results with DNA Probes: <sup>a</sup>										DA	
			LT		STh		STp		EPEC		EIEC		EHEC	
	Cases	Controls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls
1986														
November	56 (7)*	56 (8)*	0	0	NT†	NT	NT	NT	0	1	0	0	0	0
December	53 (53)	53 (52)	6	5	3	0	NT	NT	1	0	0	0	1	0
1987														
January	53 (53)	53 (52)	7	3	4	1	NT	NT	2	2	1	2	0	1
February	40 (39)	40 (38)	6	3	1	0	NT	NT	2	1	1	3	0	0
March	60 (58)	56 (54)	11	5	1	0	NT	NT	7	2	1	1	1	2
April	56 (51)	54 (53)	6	8	3	3	NT	NT	1	1	2	2	0	0
May	37 (34)	34 (34)	1	0	0	0	NT	NT	3	2	3	0	0	1
June	22 (21)	21 (21)	1	0	0	0	NT	NT	0	1	1	0	0	1
July	29 (24)	24 (23)	1	0	0	0	NT	NT	0	1	1	0	1	0
August	28 (26)	27 (25)	1	1	0	1	NT	NT	1	1	1	2	0	1
September	27 (22)	23 (20)	0	1	NT	NT	NT	NT	0	0	0	0	3	1
October	26 (24)	26 (25)	1	1	NT	NT	NT	NT	0	0	1	1	0	0
Total	487 (412)	467 (404)	41	27	12	5	17	12	13	11	6	7		
p value (one-tailed sign test)			.15		.19		.48		.5		.36			.035

\* Only lactose-positive strains have been tested so far

+ (Number of episodes where cultures were tested with DNA probes)

† NT = not yet tested

Note -- LT = heat-labile enterotoxin, STh = human heat-stable enterotoxin, STp = porcine heat-stable enterotoxin, EPEC = enteropathogenic *E. coli*, EIEC = enteroinvasive *E. coli*, EHEC enterohemorrhagic *E. coli*, DA = *E. coli* that manifest the diffuse

Table 6. Isolation of diarrheagenic E. coli in cases and controls by age group in a cohort of 360 children under prospective household surveillance from 11/1/86 to 10/31/87

Category of <u>E. coli</u>	Age Group (mos.)	Cases	Controls
<b>ETEC</b>			
LT	0 - 11	5/55*	3/53
	12 - 23	14/141	11/123
	24 - 35	12/109	7/116
	36 - 47	7/78	4/84
	≥ 48	3/30	2/30
STh	0 - 11	3/53+	1/50
	12 - 23	1/123	1/109
	24 - 35	5/93	3/99
	36 - 47	3/64	0/27
	≥ 48	0/27	0/26
<b>EPEC</b>			
	0 - 6	1/11a	1/5b
	7 - 11	7/44c	2/48d
	12 - 23	5/141	5/123
	24 - 35	3/109	3/116
	36 - 47	1/78	1/84
	≥ 48	0/30	0/30
<b>EIEC</b>			
	0 - 11	0/55	0/53
	12 - 23	6/141	5/123
	24 - 35	1/109	3/116
	36 - 47	3/78	2/84
	≥ 48	2/30	1/30
<b>EHEC</b>			
	0 - 11	0/55	1/53
	12 - 23	3/141	3/123
	24 - 35	2/109	0/116
	36 - 47	1/78	2/84
	≥ 48	0/30	1/30

\* No. positive/No. of episodes from which E. coli were tested by DNA probes.

+ Approximate denominators. So far E. coli have been tested only from the period 12/1/86 to 8/31/87.

a + c vs. b + d, p = 0.058, Fisher's exact test, 1 tail

Table 7. Admission of newborn infants into a cohort to be prospectively studied up to 24 months of age for the occurrence of diarrheal illness and to identify illness-associated bacterial, viral and protozoal pathogens

Month (1987)	Monthly Admissions	Cumulative Total of Infants	Ages (in months) of infants under surveillance:							
			< 1	1	2	3	4	5	6	7
March	12	12	12	1	2	3	4	5	6	7
April	12	24	12	12						8
May	12	36	(1)	(2)	12					
June	12	47 <sup>†</sup>	(0)	(3)	(3)	11				
July	12	59	(0)	(0)	(1)	(0)	11			
August	12	71	(1)	(3)	(1)	(1)	11			
September	12	82	(0)	(2)	(0)	(1)	(0)	11		
October	12	93	(1)	(3)	(1)	(1)	(1)	(2)	11	
			(0)	(0)	(1)	(2)	(1)	(1)	(3)	(1)
Total child months of observation:		96	84	72	59	45	34	22	11	
No. diarrheal episodes		3	13	7	5	2	4	4	1	
Incidence of diarrhea/child month:		.03	.15	.09	.08	.04	.12	.18	.09	
Total days of diarrheal morbidity:		23	110	41	32	10	31	37	5	
Mean duration of diarrheal episode:		7.7	8.5	5.9	6.4	5.0	7.8	9.3	5.0	
No. of episodes lasting > 14 days		0	2	0	0	0	0	1	0	

\* No. of infants under surveillance

† No. of episodes of diarrheal disease observed

‡ An occasional infant dropped out of the study.

**Table 8.** Preliminary data on etiologic agents identified in association with diarrheal episodes in the cohort of infants followed from birth to 24 months of age, March through October, 1987.

Pathogen	Cases			Controls		
	Age Group (mos.)			Age Group (mos.)		
	0 - 3	4 - 6	7 - 9	0 - 3	4 - 6	7 - 9
<i>Shigella flexneri</i>	0	0	0	0	0	0
ETEC	NT <sup>a</sup>	NT	NT	NT	NT	NT
EPEC	NT	NT	NT	NT	NT	NT
EIEC	NT	NT	NT	NT	NT	NT
EHEC	NT	NT	NT	NT	NT	NT
<i>Campylobacter</i>	0	1	0	0	0	0
<i>Aeromonas</i>	1	0	0	1	0	0
<i>Rotavirus</i>	1	2	0	0	0	0
<i>Cryptosporidium</i>	1	0	0	0	0	0

<sup>a</sup> NT = specimens not yet tested.

Table 9. Seasonality of isolation of *Shigella* from passive surveillance of children with diarrheal illness cultured at the Santa Julia Consultorio or at the Calvo MacKenna Children's Hospital, 11/1/86 to 10/31/87

Month	Consultorio				Hospital			
	No. Cases Cultured	Total Shigella *	S. sonnei	S. flexneri	No. Cases Cultured	Total Shigella	S. sonnei	S. flexneri
1986								
November	54	3 (5.6) <sup>+</sup>	3	0	10	2 (20)	1	1
December	102	17 (16.7)	11	5	23	6 (26)	3	3
1987								
January	128	15 (11.7)	14	0	19	6 (31.6)	5	1
February	101	10 (9.9)	8	2	12	0 (0)	0	0
March	119	10 (8.4)	4	5	13	1 (7.7)	0	1
April	77	8 (10.4)	3	5	10	1 (10)	0	1
May	49	5 (10.2)	3	1	7	1 (14.3)	0	1
June	43	1 (2.3)	0	0	2	0 (0)	0	0
July	31	3 (9.7)	0	3	7	0 (0)	0	0
August	26	4 (15.4)	1	3	4	0 (0)	0	0
September	35	1 (2.9)	0	1	3	0 (0)	0	0
October	32	2 (6.3)	0	2	8	0 (0)	0	0

\* The few isolates that were not *S. sonnei* or *S. flexneri* were *S. boydii*.

+ (Percent of cases due to *Shigella*).

**Table 10. Relative importance of Shigella species among Shigella strains isolated in the active surveillance cohort, at the consultorio or at the hospital, November 1, 1986 to October 31, 1987**

<u>Sampling Site</u>	Total	No. (%) <u>sonnei</u>	No. (%) <u>flexneri</u>	No. (%) <u>boydii</u>
	<u>Shigella</u> <u>Isolates</u>			
Active surveillance cohort	53	30 (56.6)	22 (41.5)	1 (1.9)
Passive surveillance:				
Consultorio	79	47 (59.5)	27 (34.2)	5 (6.3)
Hospital	17	9 (52.9)	8 (47.1)	0 (0)
Total	149	86 (57.7)	57 (38.3)	6 (4.0)

**Table 11. A comparison of the relative frequency of isolation of Shigella  
by sampling site and by age group,  
Santa Julia, November 1, 1986 to October 31, 1987**

<u>Sampling site</u>	<u>Age Group (mos.)</u>	<u>No. Diarrheal Episodes Cultured</u> *	<u>No. Episodes (%) due to Shigella</u>
<b>Active surveillance cohort</b>			
	0 - 11	67	3 ( 4.5)a
	12 - 23	157	11 ( 7.0)b
	24 - 35	134	13 ( 9.7)
	36 - 47	92	22 (23.9)
	≥ 48	37	4 (10.8)
<b>Passive surveillance</b>			
<b>Consultorio</b>			
	0 - 11	294	15 ( 5.1)c
	12 - 23	283	39 (13.8)d
	24 - 35	93	12 (12.9)
	36 - 47	58	6 (10.3)
	≥ 48	70	7 (10.0)
<b>Hospital</b>			
	0 - 11	95	10 (10.5)e
	12 - 23	14	4 (28.6)f
	24 - 35	7	3 (42.8)
	36 - 47	2	0 ( 0.0)
	≥ 48	0	0 ( 0.0)

\* Only one culture per child per episode was obtained for children seen at the consultorio and at the hospital. Therefore, for purposes of comparison, only the first culture from the active surveillance cohort was included in the analysis.

a vs e,  $p = 0.24$ ; a + c vs e,  $p = 0.09$ ; b vs f,  $p = 0.023$

Table 12. A comparison of the rate of isolation of Shigella from cases of diarrhea versus cases of dysentery\*

<u>Site of sampling</u>	<u>Diarrhea</u>		<u>Dysentery</u>	
	<u>Total Cases</u>	<u>No. &amp; With Shigella</u>	<u>Total Cases</u>	<u>No. &amp; With Shigella</u>
Active surveillance				
Cohort	520	64 (12.3)	47	16 (34.0)
Consultorio	798	79 (9.9)	69	21 (30.4)
Hospital	116	17 (14.7)	25	14 (56.0)
Total	1434	160 (11.2)	141	51 (36.2)

\*Dysentery is defined as the presence of frank blood and mucus in the diarrheal stool.

Table 13. Identification of diarrheagenic *E. coli* by DNA probes in coprocultures of children with diarrhea seen at Consultorio Santa Julia or hospitalized at Calvo Mackenna Children's Hospital

Month	Consultorio													
	No. Cases	No. tested	ETEC			No. Cases			No. Tested			ETEC		
			LT	STh	STp	EPEC	EIEC	EHEC	DA			LT	STh	STp
November 1986	54	0	-	-	NT	-	-	-	-	3	0	0	0	NT
December 1987	102	97	4	6	NT	7	1	0	15	20	0	0	0	NT
January	128	123	10	10	NT	3	1	0	16	18	2	2	2	NT
February	100	89	5	1	NT	7	0	0	9	14	1	0	0	NT
March	119	102	7	5	NT	10	2	0	9	13	1	0	0	NT
April	77	73	1	1	NT	6	2	0	10	10	0	0	0	NT
May	49	47	1	2	NT	2	0	0	4	7	0	0	0	NT
June	43	39	2	0	NT	0	0	1	5	2	0	0	0	NT
July	31	26	1	0	NT	2	0	0	4	7	0	0	0	NT
August	26	22	0	1	NT	1	1	0	5	4	0	0	0	NT
September	36	30	0	0	NT	0	0	1	NT	4	0	0	0	NT
October	32	30	0	0	NT	0	1	1	NT	8	0	0	0	NT

\* NT = not yet tested

Note -- Heretofore only lactose-positive strains have been tested with the DNA probes. LT = heat-labile enterotoxin, STh = human heat-stable enterotoxin, STp = porcine heat-stable enterotoxin, EPEC = enteropathogenic *E. coli*, EIEC = enteroinvasive *E. coli*, EHEC = enterohemorrhagic *E. coli*, DA = *E. coli* that manifest the diffuse adherence to HEp-2 cells. Although *E. coli* strains that manifest the diffuse adherence pattern in the HEp-2 cell assay and are positive with the DA probe are distinct from the categories of diarrheagenic *E. coli*, it is not yet clear whether or not they are indeed pathogenic.

Figure 1  
WEEKLY PREVALENCE OF SHIGELLA CARRIAGE IN 120 CHILDREN 0 - 48 MONTHS OF AGE

NO	BIRTHD	1986		1987												1988	
		M	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
1	6/85														f		
2	10/84				s												
3	3/85															f	
4	1/83														f		
5	1/83														b		
6	8/86					b											
7	1/86														f		
8	8/85	s															
9	10/84															b	
10	3/84					f											
11	1/84						f										
12	6/86																
13	7/85																
14	10/85	s//															
15	3/84					f											
16	12/83																
17	7/84					s											
18	6/83	s//															
19	4/83	s//															
20	1/84	s//															
21	4/86	f f															
22	11/86		s				s										
23	5/85	s					s										
24	11/84							s									
25	6/84	s s															
26	4/84																
27	12/82					s s									f f	//	
28	10/85		s s														
29	1/86	s															
30	3/85	f f															
31	12/84					b b											
32	7/85					s											
33	12/84																
34	5/84						s s										
35	5/83	s	s														
36	8/83					f f											
37	10/82	s s															
38	4/86		s	s													
39	5/86							s s									
40	10/85	s				f											
41	10/83	s		s													
42	12/83		s s s														
43	10/82																
44	3/83					f f f											
45	11/83																
46	12/82															b b	
47	1/84	s		s												f f	
48	2/86		s														
49	12/85		s														
50	3/84					f f f f											
51	1/84		s														
52	2/83	f	f	f		s											
53	10/83					f f f f											
54	4/84					b											
55	1/84		s	s		f f f											
56	6/85		s	s		s s s											
57	10/83	s				f f f f											
58	11/84		s b														
59	2/85	f	f f s	f	f	f	f										

f=Shigella Flexneri in the stool culture.  
s=Shigella sonnei in the stool culture.  
b=Shigella boydii in the stool culture.  
Capitals=Child has diarrhea at time of stool culture.

## APPENDIX A

### CONTRACT RELATED PUBLICATIONS AND PRESENTATIONS

#### Journal Articles

Levine MM, Ferreccio C, Black RE, Chilean Typhoid Committee, and Germanier R. Large-scale field trial of Ty21a live oral typhoid vaccine in enteric-coated capsule formulation. Lancet 1987;8541:1049-52.

Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial PA, and Levine MM. Patterns of adherence of diarrheagenic Escherichia coli to Hep-2 cells. J Pediatr Infect Dis 1987;6:829-31.

Levine MM, Ferreccio C, Black RE, Chilean Typhoid Committee, and Germanier R. New Vaccines against typhoid fever. World Pediatr Child Care 1987;3:115-26.

Levine MM, Prado V, Robins-Browne R, Lior H, Kaper JB, Moseley SL, Gicquelais K, Nataro JP, Vial P, and Tall B. DNA probes and hep-2 cell adherence assay to detect diarrheagenic Escherichia coli. J infect Dis 1988;in press.

Vial PA, Robins-Browne R, Lior H, Prado V, Kaper JB, Nataro JP, Maneval D, Elsayed A, and Levine MM. Characterization of enteroadherent-aggregative Escherichia coli, a putative agent of diarrheal disease. J infect Dis 1988;in press.

Levine MM, Ferreccio C, Black RE, Tacket CO, and Germanier R. Progress in vaccines against typhoid fever. Rev Infect Dis 1988;in press.

#### Presentations

Levine MM, Prado V, Kaper JB, Moseley S, Newland J, Tall B, Baldini M, Maggi L, Gicquelais K, and Maher K. DNA probes to detect diarrheagenic Escherichia coli. Fifth International Symposium on Rapid Methods and Automation in Microbiology and Immunology. Florence, Italy, November 1987.

Baldini MM, Gicquelais K, Maggi L, Martinez J, and Prado V. Simpler, more economical method for colony blots with biotinylated DNA probes. 88th Annual Meeting of the American Society for Microbiology. Miami Beach, Florida, May 1988.

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